Jl S. Afr. Bot. 44 (1): 83–87 (1978)

NUCLEOLAR HETEROCHROMATIN IN *ENCEPHALARTOS*

D. J. MOGFORD

(Department of Plant Sciences, Rhodes University, Grahamstown)

ABSTRACT

Using a denaturation-reannealing-fluorescence technique to illustrate the position of heterochromatin, it is shown that during interphase in the root tip cells of *Encephalartos* a close association occurs between heterochromatin and the nucleoli. The possible significance of this is discussed.

UITTREKSEL

NUKLEOLÊR HETEROCHROMATIEN IN *ENCEPHALARTOS*

Die gebruik van 'n denaturering-hergloei-fluoreserende tegniek om die posisie van heterochromatien te illustreer, toon dat daar in die wortelpunt selle van *Encephalartos* gedurende die interfase 'n nou assosiasie tussen die heterochromatien en die kernliggaampies is. Die moontlike betekenis hiervan word bespreek.

INTRODUCTION

The functions of heterochromatin, i.e. the late-replicating fraction of DNA present as condensed "chromocentres" in interphase nuclei, remain obscure. This is despite widespread application of the recently discovered fluorescence and denaturation-reannealing techniques which indicate the location of heterochromatin bands in metaphase chromosomes (Caspersson *et al.*, 1968; Pardue and Gall, 1970).

In general, heterochromatin continues to be associated with developmental effects of the quantitative type. However, great diversity occurs not only in the nature of its effects but also in the nature of heterochromatin itself.

For example, Vosa (1970) demonstrated that there exist in plants at least four types of heterochromatin, characterised variously by enhanced or reduced quinacrine fluorescence and by positive or negative sensitivity to cold. The various responses of heterochromatin to denaturation-reannealing treatment followed by Giemsa staining add further complexity to the situation, such that in *Allium flavum* alone there exist four types of heterochromatin as characterised by their various responses to fluorescence and Giemsa staining (Vosa, 1973).

Of late, it has proved possible to obtain new evidence on the functions of heterochromatin by using the new techniques to identify the location of heterochromatin in interphase nuclei. Thus Mogford (1977) demonstrated that heterochromatin is involved in an end-to-end fusion of chromosomes in onion

---

Accepted for publication 14th July, 1977.

interphase nuclei, with the various consequences this implies for gene transcription and meiotic pairing.

The present study was designed to investigate the relationship between heterochromatin and nucleolar synthesis in the interphase nuclei of *Encephalartos* species.

MATERIAL AND METHODS

Investigations were performed on growing root tips from seedling specimens of *Encephalartos lehemanni* Ecklon ex Lehm. and *E. caffer* Miq.

Nucleoli were studied by means of phase-contrast illumination using visible light, while heterochromatin was revealed using the combined denaturation-reannealing-fluorescence technique described previously (Mogford, 1977). The relationship between heterochromatin and the nucleoli was observed by means of simultaneous reflected-light fluorescence and transmitted-light phase contrast, using a Zeiss fluorescence microscope.

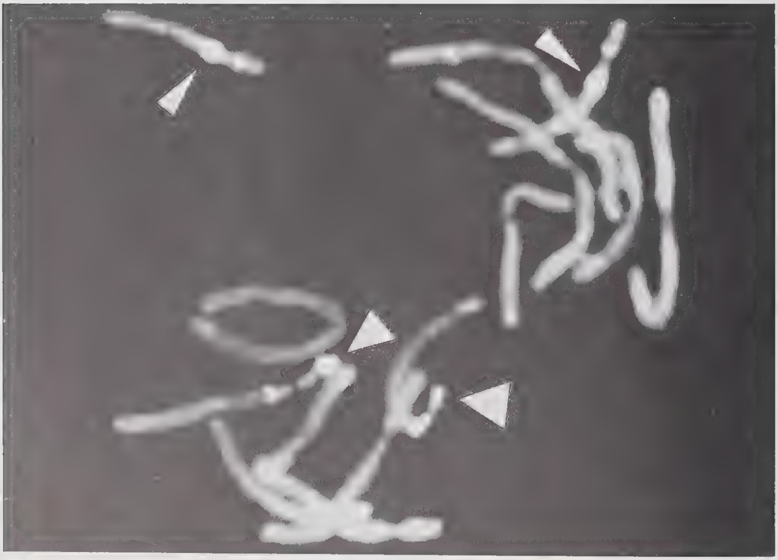

FIG. 1.

Metaphase chromosomes of *E. lehemanni*, prepared as indicated in text. Arrows indicate positions of heterochromatin; note the heterochromatic (and heteromorphic) satellites of the nucleolar organising chromosomes, marked by the larger arrows. X1349.

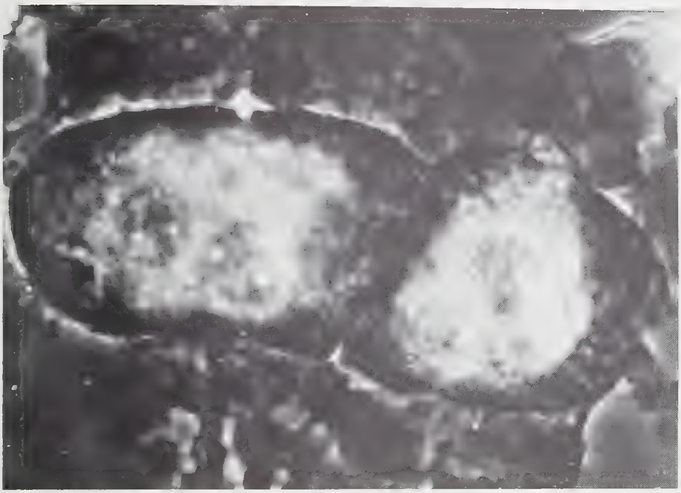

FIG. 2.
E. lehemanni, X1349.

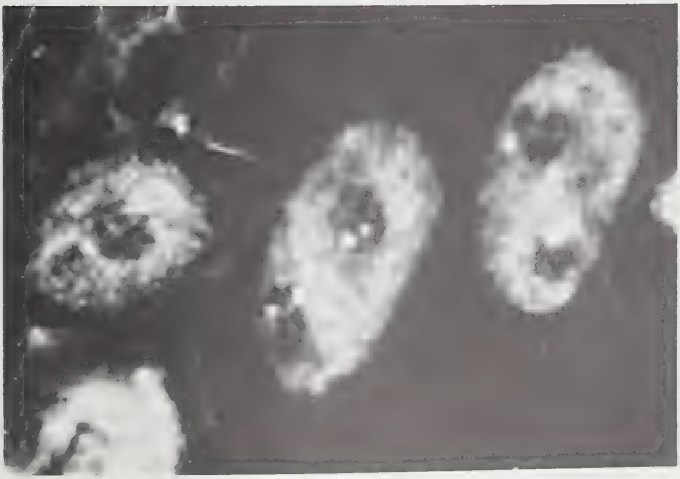

FIG. 3.
E. caffer, X843.

FIGS 2 & 3
Interphase nuclei of *Encephalartos*, showing relationship between heterochromatin and nucleoli. Photographed using combined fluorescence and phase contrast as described in text.

RESULTS

Heterochromatin in *E. lehemanni* was found to be present on the nucleolar chromosome pair, principally in the vicinity of the nucleolar organisers, and also on certain of the other chromosomes (Fig. 1).

In both species, the heterochromatin visible during interphase was found to be strongly localised at positions along the borders of the nucleoli (Figs 2 & 3). It was assumed that this heterochromatin was that of the nucleolar chromosome pair, though the possibility that other heterochromatin was involved as well could not be ruled out since fusion of chromocentres during interphase is a common phenomenon.

DISCUSSION

The occurrence of heterochromatin in the vicinity of the nucleolar organisers, even in the quantities recorded in the present observations, is not by itself unusual. What is unusual is the extremely conspicuous association of heterochromatin with nucleoli in the interphase nuclei. This association in cycads may to some extent be inferred by conventional staining (Marchant, 1968), but is demonstrated with particular clarity by the present technique.

The relationship raises again the possible involvement of heterochromatin in nucleolar synthesis, for the consistency of the association is greater than that which might be expected simply from the location of heterochromatin in the vicinity of the secondary constrictions.

Brown (1966) suggested that heterochromatin in the vicinity of the nucleolar organiser might serve as an inert region of chromosome, separating off the intense ribosomal RNA synthetic activity of the nucleolus organiser itself from the activities of the remainder of the chromosome.

An alternative possibility is that heterochromatin itself might be involved in nucleolar synthesis. Certainly, such an intense and specific role would be consistent with the high degree of base repetition which modern studies indicate as characterising heterochromatin. Such repetition, indeed, would be consistent with the general quantitative action of heterochromatin. The difficulty here is that DNA in the heterochromatic state is normally assumed to be non-synthetic, though as a generalisation this is now less tenable in view of recent evidence for a differential amplification of certain heterochromatic DNA in both plant and animal species (Schweizer and Nagl, 1976). Of particular relevance is the observation of a differential amplification of nucleolus-associated heterochromatin in both the beetle *Dytiscus marginalis* and the cricket *Acheta domestica* (John and Lewis, 1975). The possibility of a synthetic role cannot therefore be excluded.

ACKNOWLEDGMENT

May I thank Mr Cameron of the Grahamstown Botanical Garden for the loan of cycad seedlings.

REFERENCES

BROWN, S. W., 1966. Heterochromatin. *Science, N.Y.* **151:** 417–425.

CASPERSSON, T., FARBER, S., FOLEY, G. E., KUDYNOWSKI, J., MODEST, E. J., SIMONSSON, E., WAGH, U. and ZECH, L., 1968. Chemical differentiation along metaphase chromosomes. *Expl. Cell Res.* **49:** 214–222.

JOHN, B. and LEWIS, K. R., 1975. *Chromosome hierarchy*. Oxford University Press.

MARCHANT, C. J., 1968. Chromosome patterns and nuclear phenomena in the cycad families *Stangeriaceae* and *Zamiaceae*. *Chromosoma* **24:** 100–134.

MOGFORD, D. J., 1977. Chromosome associations in onion root tip nuclei. *Jl S. Afr. Bot.* **43:** 97–102.

PARDUE, M. L., and GALL, J. C., 1970. Chromosome localisation of mouse satellite DNA. *Science* **168:** 1356–1358.

SCHWEIZER, D., and NAGL, W., 1976. Heterochromatin diversity in *Cymbidium*, and its relationship to differential DNA replication. *Expl. Cell Res.* **98:** 411–423.

VOSA, C. G., 1970. Heterochromatin recognition with fluorochromes. *Chromosoma* **30:** 366–372.

VOSA, C. G., 1973. The enhanced and reduced fluorescence bands and their relationship to the Giemsa patterns in *Allium flavum*. *Nobel Symposium* **23:** 156–158.